BIS (AMINO ACID) MOLECULAR SCAFFOLDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to the Traditional Application entitled "*BIS* (AMINO ACID) MOLECULAR SCAFFOLDS", filed July 2, 2003 in the name of Christian E. Schafmeister and, under 35 USC 119(e), to provisional application Serial No. 60/401,474, filed August 6, 2002, both expressly incorporated herein by reference.

FIELD OF THE INVENTION

The present invention provides molecular building blocks of rigid *bis*(amino acids). The molecular building blocks can be linked together through the formation of rigid diketopiperazine rings, to provide the desired three dimensional structure.

BACKGROUND INFORMATION

Biological proteins and catalytic RNA's are nature's general solution to the problem of how to construct nanoscale molecular devices. The powerful catalytic, information processing and energy transduction capabilities of proteins are examples of the powers inherent in molecules that are large enough to encapsulate smaller molecules and structured enough to position functional groups in three-dimensional space. Despite the thousands of high-resolution structures of biological proteins that have been determined and the several decades of effort from the computational biology community, the protein folding problem is still not well understood. Given an arbitrary primary sequence of a protein, the structure it will assume once it folds cannot be predicted. The converse problem, predicting the primary sequence of a protein that has a desired structure is also poorly understood. The *de novo* design of unnatural proteins is a very active area of research[1, 2] and has yielded several significant advances in the *de novo* design of simple α-helical and β-sheet proteins. But beyond very simple protein folds[3], the systematic construction of unnatural functional proteins is still not possible. The synthesis of poly-peptides both by chemical and biological means has become straight-forward, the harder problem is predicting how poly-peptides will fold.

Synthetic approaches to the synthesis of constitutionally precise macromolecules have been developed. Organic chemists are able to synthesize very large natural products, such as brevetoxin B [4-6]. However, as the size of a synthetic target increases, the amount of labor that is required to construct the molecule becomes prohibitive. Several groups have developed systematic approaches to the synthesis of constitutionally defined macromolecules such as Michl's "molecule sized Tinkertoy construction set" [7], Rebek's molecular capsules [8], Stoddart's "Molecular meccano kit" [9], and the supramolecular work of Jean-Marie Lehn [10], to name a few. These projects create molecules that are either flexible because they contain many freely rotating covalent bonds[7, 9] or they consist of supramolecular clusters held together by many weak non-covalent bonds[8-10]. Dendrimers and non-natural polymers are molecules with high molecular weights, but these molecules are also either highly flexible, highly symmetric or both [11]. Cyclophanes and cyclodextrins [12] are structurally well defined oligomers that attain a higher degree of structure than linear oligomers because they are constrained to form rings. These basket shaped molecules have generated a great deal of scientific interest as receptor and enzyme mimics but they are limited in the cavity sizes and shapes that they can form and in the complexity of the binding surface that they can present to guests.

New approaches to structured macromolecules such as the poly-β-peptide approach of Gellman and Seebaeh signify a tremendous advance [13-15]. These oligomeric molecules adopt complex secondary structure with as few as six residues. However, in order to create β-peptides with complex tertiary structure the folding rules of β-peptides will first need to be elucidated. The folding of β-peptides will rely on many weak non-covalent interactions to define tertiary structure. There is a need for molecular building blocks which can be linked together to create a pre-defined and desired shape.

SUMMARY OF THE INVENTION

The present invention provides a new approach for synthesizing constitutionally precise macromolecules that will be used to design functional nanoscale molecular devices. The approach involves the coupling of synthetic stereochemically pure monomers through amide bonds to form oligomers in a manner similar to the synthesis of poly-peptides and poly-β-peptides. While traditional

polypeptides and poly-β-peptides are assembled from flexible amino acids coupled through single amide bonds, the *bis*-peptides of the present invention are assembled from cyclic bis-amino acids that are coupled through pairs of amide bonds. This approach avoids the need to solve difficult folding problems because rigid ladder and spiro-ladder oligomers that contain no freely rotating bonds, within the core structure of the oligomer, are synthesized. Each monomer has a cyclic or fused ring structure and contains multiple stereocenters. Each monomer holds its two partners in a well defined orientation and distance with respect to each other. By assembling monomers in different sequences, an enormous number of macromolecules with different three-dimensional structures can be constructed (Figure 2). These macromolecules can be used as scaffolds to present chemically reactive groups to carry out designed functions.

In contrast to previous methods, the present invention avoids the use of freely rotating covalent bonds in molecular scaffolds and reliance on the use of non-covalent interactions to maintain tertiary structure. This approach creates molecules that are highly structured and highly asymmetric, and can create cavities with an enormous variety of shapes and sizes while presenting a wide range of functional groups.

The molecular scaffolds can satisfy Cram's requirements of pre-organization and complementarity to act as hosts. An important requirement of macromolecules that will display biomimetic function is that they be capable of acting as hosts for small molecules. Pre-organization enhances binding by reducing the enthalphic cost of reorganization and the entropic cost of ordering the receptor on binding its guest. Molecular recognition occurs when the guest attaches non-covalently to the host, and is driven by a reduction of free energy associated with the formation of many complementary hydrophobic and electrostatic contacts between the host and guest. Each complementary contact contributes a very small amount of stabilization to the overall interaction. Every binding interaction must involve an interaction free energy of several times kT in order to at least overcome the loss of rotational and translational entropy of about 4.5-6.0 kcal/mole [16] when the complex forms. Many biological hosts almost completely encapsulate their guests because many weak complementary contacts must work together to create a strong interaction.

A molecular host is an orderly array of functional groups, maintained in a pre-organized arrangement in three-dimensional space around a cavity [17]. In aqueous solution, functional groups include hydrophobic groups, hydrogen bonding groups, charged groups, aromatic groups and so on. In organic solvents, hydrogen bonding becomes very stabilizing and solvophobic effects are weaker. The molecular scaffolds of the present invention are highly ordered but complex macromolecules fully capable of displaying pre-organization as described by Cram [18]. They can also display cavities that contain several functional groups and thus should be fully capable of displaying the property of complementarity to desired guests.

This approach complements and offers advantages over other synthetic approaches to macromolecules. Many research groups have designed hosts and demonstrated binding to guests. Wilcox and co-workers have designed a minimalist host that displays two carboxylic acid groups and binds adenine and biotin derivatives strongly in organic solvents but weakly in methanol-water mixtures [19]. Diederich and co-workers have constructed a porphyrin-bridged cyclophane that binds arenes and catalyzes their oxidation with a turn-over number of about fourteen in methanol-d4/D2O/acetie acid (95:4.85:0.15% v/v) mixtures. Rebek and co-workers have made many host molecules including self-assembling capsules[20] that dimerize and bind Boc protected amino esters in mesitylene D_{12} solvent. All of these functional molecules are assembled with short syntheses. The Rebek capsule is synthesized in two steps, the Wilcox molecule in four steps, and the Diederich molecule in about twelve steps. The building blocks of the present invention are synthesized in eight to fourteen steps, and will be assembled on solid support or in solution in a multi-step synthesis involving two steps for every building block.

The molecular scaffold methodology of the present invention is significant because it offers a systematic approach to the construction of macromolecules with precise control over size, shape, chemical and mechanical properties. This will have great future value in the design of nanoscale devices and macromolecules with biomimetic function.

It is an object of the present invention, therefore, to provide molecular building blocks which can be assembled into discrete shapes.

It is an additional object of the present invention to provide molecular building blocks made from *bis* (amino acids).

It is a further object of the present invention to provide a method of synthesis of *bis* peptides, using the *bis* (amino acid) molecular building blocks.

These and other objects will become more readily apparent from the following detailed description, drawings, and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is further illustrated by the following non-limited drawings in which:

Figure 1 : The general structures of nine classes of *bis*-amino acid molecular building blocks.

Figure 2 : A two-dimensional diagram illustrating the approach to macromolecule construction.

Figure 3 : The two phases of scaffold synthesis, elongation followed by rigidification.

Figure 4 : The four building blocks in the *pro4* monomer class.

Figure 5 : The crude C_{18} HPLC chromatogram of the five-mer (NH2-*Tyr*-**1**:*pro4 (2S4S)5*).

Figure 6 : An alphabet soup of predicted structures for headed arrows represent expected close contacts that will NMR spectra.

Figure 7 : The time dependant product formation of scaffolds containing "n" diketopiperazines.

Figure 8 : The four members of the *pro3* monomer class.

Figure 9 : The four accessible stereoisomeric members of the *pro4a* monomer class. "R" is a suitable functional group.

Figure 10 : Various amines that could be coupled to 75 to create functionalized monomers.

Figure 11: the reaction scheme for **1**:*pro4(2S4S)*

Figure 12: the reaction scheme for **2**:*pro4(2S4R)*

Figure 13: the reaction scheme for **3**:*pro4(2R4R)* and **4**:*pro4(2R4S)*.

Figure 14: the reaction scheme for synthesis of the three-mer scaffold **19**.

Figure 15: the reaction scheme for synthesis of the five-mer scaffold **33**.

Figure 16: the reaction scheme for synthesis of **44**.

Figure 17: the reaction scheme for synthesis of **35**.

Figure 18: the reaction scheme for synthesis of *pro3(2R3S)* and *pro3(2R3R)*.

Figure 19: reaction schemes for synthesis of the *pip4* and *pip5* classes.

Figure 20: the reaction scheme for synthesis of the *hin* class.

Figure 21: the reaction scheme for synthesis of **72** and **73**.

Figure 22: the reaction scheme for synthesis of **76**.

Figure 23: the reaction scheme for synthesis of *pro4a(2S3R4S)NHR*.

Figure 24: the reaction scheme for synthesis of *pro3r(2S3S)*.

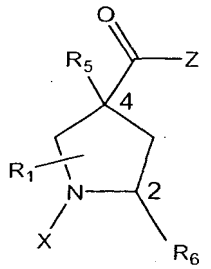
Figure 25: Sequence of three-mer **19**.

Figure 26: Sequence of compounds **21**, **22**, **23** and **24**.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the present invention provides *bis* amino acids, that have been suitably modified to enable them to carry out the diketopiperization reaction, described more fully below, to form the rigid macromolecule of interest. As used herein, the term "*bis* amino acid" refers to any suitably modified amino acid, as exemplified by the structures below, that can provide the necessary reactivity for the diketopiperization reaction. Suitable *bis* amino acids include, but are not limited to, those structures specifically defined below.

In one embodiment, the present invention provides compounds having the formula



(1)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3 or 5;

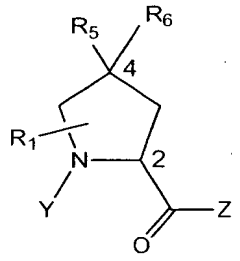
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 4 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(2)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3 or 5;

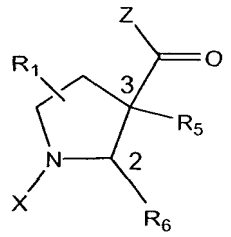
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 4 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(3)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 4 or 5;

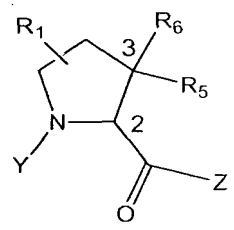
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 3 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In another embodiment, the present invention provides compounds having the formula



(4)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 4 or 5;

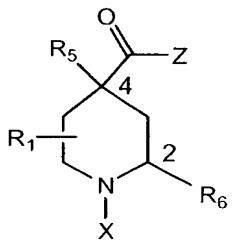
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 3 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(5)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 5 or 6;

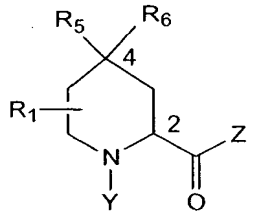
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 4 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(6)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 5 or 6;

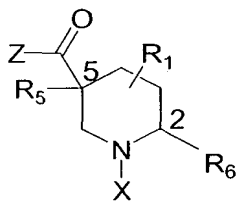
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 4 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(7)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4 or 6;

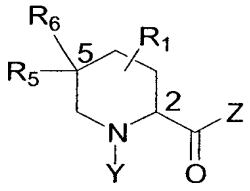
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 5 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(8)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 5 or 6;

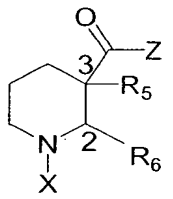
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 5 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(9)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 4, 5 or 6;

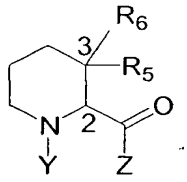
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 3 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(10)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 4, 5 or 6;

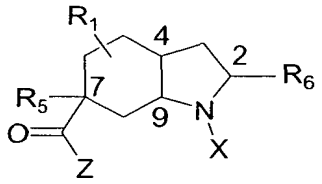
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2 and 3 and of the carbon bearing R_1 (if R_1 is not H) can be any one of (S,S,S), (S,S,R), (S,R,S), (S,R,R), (R,S,S), (R,S,R), (R,R,S) or (R,R,R).

In an additional embodiment, the present invention provides compounds having the formula



(11)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5, 6, 8 or 9;

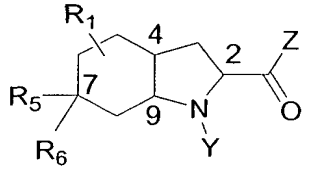
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2, 4, 7, 9 and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(12)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5, 6, 8 or 9;

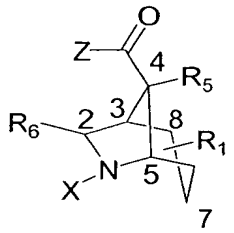
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at positions 2, 4, 7, 9 and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(13)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 5, 6, 7 or 8;

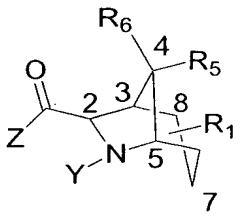
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 4 and 5, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(14)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 5, 6, 7 or 8;

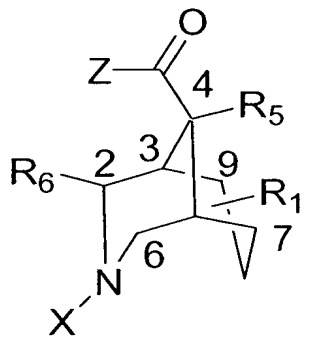
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 4 and 5, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(15)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 5, 6, 7, 8 or 9;

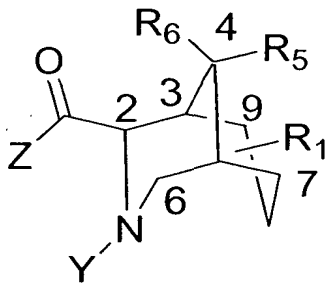
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 4 and 5, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(16)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 5, 6, 7, 8 or 9;

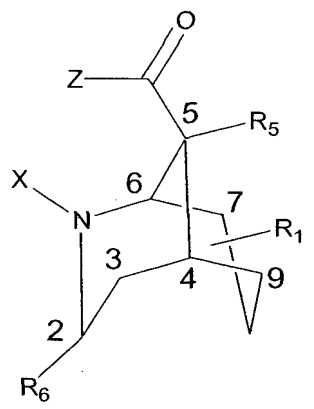
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 4 and 5, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(17)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 6, 7, 8 or 9;

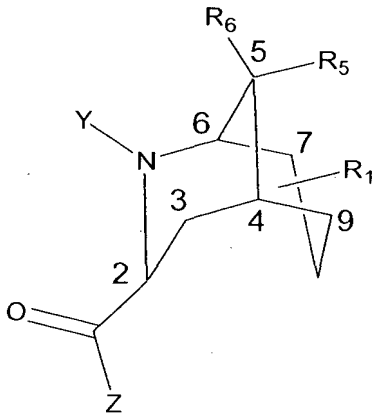
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 4, 5 and 6, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(18)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 6, 7, 8 or 9;

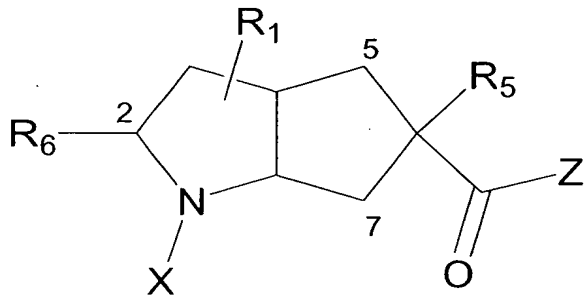
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 4, 5 and 6, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(19)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5, 7 or 8;

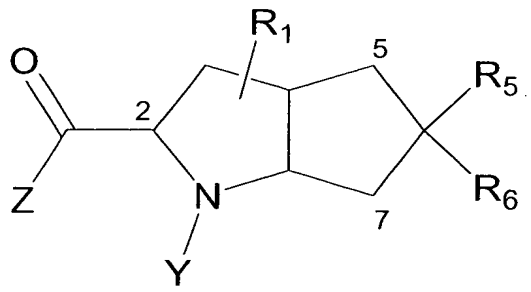
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 4, 6 and 8, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(20)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5, 7 or 8;

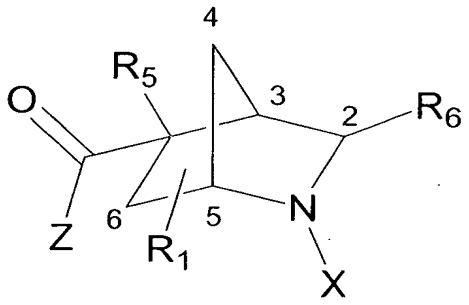
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 4, 6 and 8, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(21)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5 or 6;

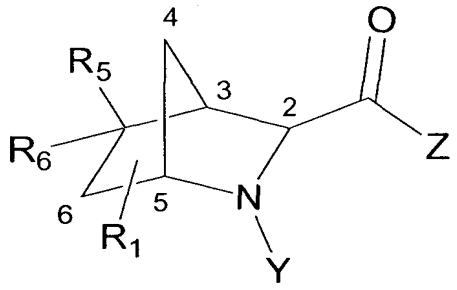
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 5 and 7, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(22)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5 or 6;

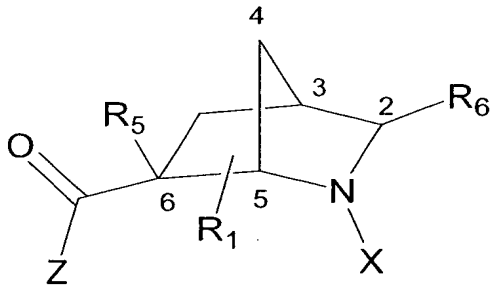
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 5 and 7, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(23)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5 or 7;

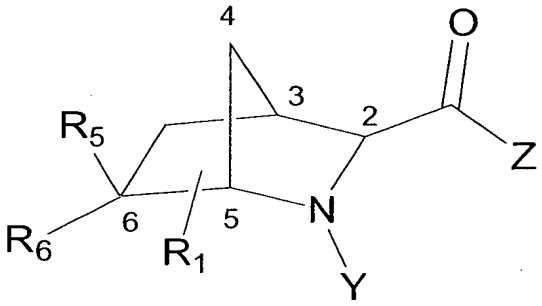
R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2Y;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 5 and 6, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

In an additional embodiment, the present invention provides compounds having the formula



(24)

where:

X represents a first amine protecting group;

Y represents a second amine protecting group;

Z represents a weak leaving group;

R_1 represents an H, or a functional group, and can be attached to the molecule at positions 2, 3, 4, 5 or 7;

R_2 represents an H or a functional group;

R_5 represents N_3 or NR_2X;

R_6 represents a carboxylic acid or a strongly activated ester ; and

the stereochemical configuration at the positions 2, 3, 5 and 6, and of the carbon bearing R_1 (if R_1 is not H) can be any of the 32 combinations of (R) and (S).

As used herein, the term "amine protecting group" refers to a moiety that protects the nitrogen of interest from attack during synthesis, and which can be easily removed at a later stage during formation of the desired compound of interest. Protecting groups are well known in the art, and include, for example, the protecting groups described in the book "*Protective Groups in Organic Synthesis*" by Theodora W. Greene and Peter G. M. Wuts, John Wiley and Sons publisher. Any suitable amine protecting group can be used. Preferred amine protecting groups include compounds such as 9-fluorenylmethyl carbamate, allyl-carbamate, benzyl-carbamate, substituted benzyl-carbamates, t-Butyl carbamate, 1-adamantyl carbamate, 2-nitrobenzenesulfonyl, triphenylmethyl, and (4-methoxyphenyl)diphenylmethyl and 9-phenylfluorenyl and the like. Preferably, two different amine protecting groups will be used, but under certain conditions it may be desirable to use the same amine protecting group in a given synthesis.

As used herein, the term "weak leaving group" refers to those moieties that can protect the carboxylate from attack when the monomer is first coupled to the scaffold, and leaves only upon rigidification. Such leaving groups are well known in the art. Preferably, the leaving group will mildly activate the carboxylate, so that when the monomer is in the completed chain, the nearest amine will attack the carboxylate spontaneously to form the diketopiperazaine bond. Any suitable leaving group can be used, and non-limiting examples include short chain alkoxides, thiolates, azide and sulfonamides. Thiolates include thiolates formed from short chain thiols such as ethanethiol, methanethiol, thiophenol and substituted thiophenols. Preferred weak leaving groups include fragments such as alkoxides formed from methanol, ethanol, 2-fluoroethanol, 2,2-difluoroethanol, 2,2,2-trichloroethanol, 2,2,2-trifluoroethanol, phenol, and substituted phenols.

As used herein, the term "strongly activated ester" will be used to refer to esters such as a pentafluorophenyl ester (-COOPfp), N-hydroxysuccinimide ester (-COONHS), symmetric anhydrides or asymmetric anhydrides, or other similar esters, that are rapidly attacked by amines in a bimolecular reaction under the typical conditions used in amide bond formation..

As used herein, the term "functional group" refers to any moiety that can provide additional functionality beyond that provided in the basic monomer. As an analogy, each naturally occurring amino acid contains a functional group that confers a unique set of properties, such as size, reactivity, charge, and the like, on the side-chain of each amino acid. Any functional group can be used, to confer the desired properties, so long as the use of such group does not interfere with oligomer formation. Suitable functional groups include, for example, but are not limited to, an alkyl group, a lower alkyl group, an alkoxy group, a cycloalkyl, a heterocycloalkyl, an aryl group, a heteroaryl group, an alkoxyaryl group, an aralkyl group, an aralkoxy group, an alkylthiogroup, an arylthiogroup, an alkylamido group, an alkylsulfinyl group, an alkylsulfonyl group, an alkacyl group, an alkylsulfoxide group, a halogen and a nitro group, as those terms are understood in the art.

The term "alkyl" refers to straight and branched chain alkyl groups having one to 50 carbon atoms. The term "lower alkyl" refers to an alkyl having from 1 to 8 carbon atoms.

The term "cycloalkyl" refers to saturated carbocycles having from three to twelve carbon atoms, including bicyclic and tricyclic cycloalkyl structures. A "hetero-cycloalkyl" group refers to a monocyclic radical containing carbon atoms, preferably 4 or 5 ring carbon atoms, and at least one heteroatom selected from nitrogen, oxygen and sulfur, and having no unsaturation.

The term "aryl" and "heteraryl" refer to monocyclic and polycyclic unsaturated or aromatic ring structures, with "aryl" referring to those that are carbocycles and "heteraryl" referring to those that are heterocycles. Such moieties may be optionally substituted with on or more suitable substituents, for example, a halogen, a lower alkyl (C_1-C_8), OH, NH_2, CN, COOH, O-lower alkyl, and the like.

Any of the above functional groups can optionally be substituted with any suitable substituent, including a halogen, lower alkyl group, -aryl, -OH, -NO_2, -CN, -

CO_2, -O-lower alkyl, and the like. This list is non-limiting, and any substitution can be used, provided that the substitution does not interfere with the ability of the functional group to confer the desired properties on the monomer.

As can be seen in the above formulas, none of the compounds described have two carboxylic acids. It is important to have one and only one carboxylic acid (-COOH) or strongly activated ester such as a pentafluorophenyl ester (-COOPfp), an N-hydroxysuccinimide ester (-COONHS), a symmetric or an asymmetric anhydride. This is to enable the building block to be coupled to a growing macromolecule in the synthesis of *bis*-peptides. *Bis*-amino acids with two stable esters are unable to couple and useless for the synthesis of *bis*-peptides. *Bis*-amino acids with two carboxylic acids or two strongly activated esters will couple indiscriminately through both groups and create useless mixtures of products.

The present invention provides a collection of *bis*-amino acids that are the molecular building blocks of a unique methodology. Each building block has a unique rigid three-dimensional structure and contains multiple stereocenters (each indicated with "*" in Figure 1). The building blocks are grouped in classes. The members of a class have identical constitution but vary in their stereochemistry. Each class contains at least two stereoisomers that must be synthesized or isolated in stereochemically pure form. Many of the *bis*-amino acids shown can be synthesized by following the same basic strategy. The strategy is to start from a stereochemically pure cyclic intermediate that contains a protected *a*-amino ester and a ketone. The ketone may then be converted to a hydantoin using a Bucherer-Bergs reaction and the hydantoin is then hydrolyzed and converted into another suitably protected amino ester. The Bucherer-Bergs reaction produces at most a mixture of two diastereomers that can be separated and carried individually through to form two valuable diastereomeric building blocks. A modified Corey-Link reaction[21] may also be used to convert the ketone into an azido-ester that is equivalent to a protected amino ester. The modified Corey-Link reaction also creates a mixture of two diastereomers that are separated and carried individually through to form two valuable diastereomeric building blocks. This strategy is non limiting, many other approaches can be envisioned for the synthesis of bis-amino acid building blocks.

Ideally, scaffold assembly takes place in two phases (Figure 3). First, the building blocks are coupled on solid support through amide bonds as a flexible chain in an "elongation phase". Then the chain is cleaved from solid support and a second set of amide bonds are formed in parallel in the "rigidification phase". The result is a spiro-ladder oligomer with a complex and well defined three-dimensional shape that is determined by the sequence of its monomers. Solid phase peptide synthesis has made it possible to routinely synthesize peptides with lengths in excess of 40 amino acids with excellent yields[28]. Similarly, it is possible to synthesize molecules with defined shapes in the range of 1,000 to 10,000 Da. Making ten-mers with just four building blocks, 4^{10} or about 1,000,000 different rigid macromolecular shapes can be constructed. The synthesis of every one of these million different molecules will be quick and will follow exactly the same synthetic steps (but using different building blocks) on solid support. Alternatively, scaffolds can be assembled in solution. "Rigidification" can also be carried out at the same time the chain is being elongated.

As will be known to one skilled in the art, the process of optimizing the synthesis of the *bis*-amino acid monomers involves modifying reaction conditions to improve yields, modifying workup conditions to improve purity and recovery of products, eliminating chromatographic steps, developing crystallization procedures to isolate pure products and developing alternative routes to intermediates that reduce cost and improve yields of the final *bis*-amino acid products.

In an additional aspect, the present invention provides a method of synthesizing *bis* peptides comprising the steps of:

1) providing a solid support;

2) activating a first *bis* amino acid or naturally occurring amino acid;

3) attaching the *bis* amino acid or naturally occurring amino acid to the support;

4) removing the leading edge amine protecting group if a *bis* amino acid is used, or the amine protecting group if a naturally occurring amino acid is used;

5) activating and attaching a next *bis* amino acid or a next naturally occurring amino acid to the leading edge amine of the *bis* amino acid or amine of the naturally occurring amino acid; and

6) repeating steps 4 and 5 as necessary to achieve the desired chain length;

7) detaching the synthesized *bis* peptide from the support; and

8) isolating the synthesized *bis* peptide,

where the *bis* peptide synthesized in the above manner has at least two contiguous *bis* amino acids, and a rigidification step is carried out either after step 4 or after detachment of the *bis* peptide from the solid support. Optionally, the method further comprising the step of modifiying or adding a functional group, after step 5. Each of these steps is more fully described below.

In the synthesis of *bis*-peptides, natural and unnatural amino acids can be introduced into the bis-peptide structure to confer additional properties. Only those macromolecules, that can be synthesized by this process, that contain at least two contiguous *bis*-amino acids and undergo rigidification to form a pair of bonds between the two contiguous *bis*-amino acids, are considered within the scope of the present invention.

Bis-peptide synthesis on solid support takes place by the following process:

A suitable solid phase support is prepared or purchased, such as a polystyrene resin purchased from Novabiochem for solid phase synthesis of peptides and organic compounds. Other resins can also be used. A wide variety of linkers can be used, preferred linkers are those derived from 4-hydroxymethylpheoxyacetic acid (HMPA), linkers derived from 4-hydroxymethylbenzoic acid (HMBA), RINK linkers, silylalkyl linkers, and 4-sulfamylbenzoyl based linkers.

If the resin/linker combination carries a temporary protecting group, it is removed using appropriate reaction conditions.

A protected *bis*-amino acid is chosen from the collection of monomers described above or a protected amino acid is chosen; this chosen monomer is activated and coupled to the solid support. Any of the *bis* amino acids of the present invention, or naturally occurring amino acids, can be used, and are within the scope of the present invention. If the monomer is in a pre-activated form, such as a pentafluorophenyl ester, it is first dissolved in a suitable solvent and base is added after which the mixture is added to and incubated with the resin. If the bis-amino acid is in the free-acid form, an activating agent is added with solvent and base and then the mixture is added to and incubated with the resin. Preferred solvents are N,N-dimethylformamide, 1-methyl-2-pyrrolidinone, N,N-dimethylacetimide and

methylene-chloride in an appropriate ratio to ensure solubility. Preferred bases are diisopropylethylamine and triethylamine. As used herein, the term "activating agent" refers to those activating agents known to one skilled in the art and used in peptide synthesis. These are available, for example, from Novabiochem. Preferred activating agents are those that lead to the formation of 1-hydroxy-7-azabenzotriazole (HOAt) esters, N-hydroxysuccinimide (HOSu) esters, N-hydroxybenzotriazole esters (HOBt), acid fluorides. Other activation reagents such as benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphate (BOP), 1,1-carbonyl-diimidazole (CDI), N,N-dialkylcarbodiimides, bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP) can also be used. An excess of activated monomer is used to ensure a high yield of coupled product. Typically two to four equivalents are added. Sufficient time is allowed for the coupling to take place, typically 10 to 120 minutes.

The resin is then subjected to several wash cycles to remove byproducts and to prepare the resin for the next operation. The preferred method of washing is three five minute cycles of a solvent swelling solvent such as N,N-dimethylformamide followed by three five minute cycles of a solvent shrinking solvent such as isopropanol or methanol, followed by three five minute cycles of the solvent swelling solvent.

At this stage, the decision may be made to modify the monomers on the resin to attach additional functionality or modify functionality. Preferred modifications are alkylation of sulfonamide protected amines or oxidation, reduction, alkylation, acylation or amidation of the appended R_1 functional group described in the monomer claims. Adding a functional group can be carried out after the adddition of each new monomer, as desired.

The leading edge amine protecting group is removed from the most recently attached monomer. The resin is subjected to reaction conditions appropriate to remove the temporary protecting group without cleaving the growing chain from the resin. If the leading edge amine is masked as an azide, the azide is reduced to liberate the amine.

If the monomer is a bis-amino acid then the temporary protecting group on the leading edge amine is removed using conditions appropriate for the temporary protecting group. The leading edge amine is defined as follows. Each monomer

carries two protected alpha-amino acids. The leading edge amine is the amine on the monomer that is not directly connected to the carbon alpha to the activated ester is now attached to the resin. In other words, the leading edge amine is the amine alpha to the carbonyl carrying the weak leaving group.

If the monomer is a regular protected amino acid then the temporary protecting group is removed from the amine.

The resin is then subjected to several wash cycles to remove byproducts and to prepare the resin for the next operation. The preferred method of washing is three five minute cycles of a solvent swelling solvent such as N,N-dimethylformamide followed by three five minute cycles of a solvent shrinking solvent such as isopropanol or methanol, followed by three five minute cycles of the solvent swelling solvent.

The next monomer is activated and attached, as described above, with as many repetitions of these steps as necessary to achieve the desired chain length.

At this stage the decision may be made to rigidify one or more of the contiguous bis-amino acids in the growing chain while it is still attached to the resin. This would be carried out by removing the protecting group from the trailing edge amine (the amine connected to the alpha carbon shared by the carbonyl that was first attached to the resin). After removal of the protecting group, the resin is incubated under either neutral, basic or acidic conditions to promote the multiple, parallel, intramolecular aminolysis reactions that rigidify the scaffold. As used herein, the terms "rigidify" or "rigidification" will be used to refer to this two-step process.

The activation and attachment steps are repeated for every monomer that is to be incorporated into the growing macromolecule. As the final monomer is coupled, the decision may be made to leave the last leading edge amine protecting group on the resin during cleavage.

Once the last monomer has been added, the decision may be made to attach a final group to the resin through the last leading edge amine. Examples of useful modifications would be to treat the resin with dansyl-chloride to attach a fluorescent dansyl group to the resin, or to treat the resin with activated carboxylic acids.

At this stage the decision may be made to rigidify one or more of the contiguous *bis*-amino acids in the growing chain while it is still attached to the resin. This would be carried out by removing the protecting group from the trailing edge

amine (the amine connected to the alpha carbon shared by the carbonyl that was first attached to the resin). After removal of the protecting group, the resin is incubated under either neutral, basic or acidic conditions to promote the intramolecular aminolysis reactions that rigidify the scaffold.

The new macromolecule is then cleaved from the solid support using cleavage conditions appropriate for the linker/resin on which the scaffold was assembled. The macromolecule will then be isolated and if necessary, purified using reverse phase high pressure liquid chromatography. A preferred method of isolation of the macromolecule from cleavage byproducts is to rapidly dilute the cleavage mixture into diethyl ether and isolate the product by centrifugation. A preferred situation is to use an acid cleavable linker and acid cleavable protecting groups on the trailing edge amines. Strong acid cleavage conditions such as hydrogen fluoride or trifluoromethanesulfonic acid will simultaneously cleave the macromolecule from the resin and strip all of the acid cleavable protecting groups from the macromolecule preparing it for rigidification by incubation under conditions that promote intramolecular aminolysis.

Bis-amino acids that require rigidification at this stage and where the trailing edge amine protecting groups have not been removed under the resin cleavage conditions, will be rigidified by removing the protecting group from the trailing edge amine (the amine connected to the alpha carbon shared by the carbonyl that was first attached to the resin). After removal of the protecting group, the resin is incubated under either neutral, basic or acidic conditions to promote the intramolecular aminolysis reactions that rigidify the scaffold. For example, if Cbz groups are used as the trailing edge amine temporary protecting group and they have not been removed by strong acid cleavage of the linker, they can be removed at this point using hydrogenolysis with hydrogen gas on palladium catalyst. A preferred method of promoting diketopiperazine formation through intramolecular aminolysis is to incubate the macromolecule in 20% piperidine in dimethylformamide for 48 hours. Basic conditions are known to promote epimerization of diketopiperazines so care must be taken. Slower, acidic conditions can also be used and they are known to avoid epimerization of diketopiperazines.

The rigidified scaffold is isolated and may be purified by reverse phase high pressure liquid chromatography. The preferred method of isolating the rigidified scaffold is by rapidly diluting a solution of the macromolecule into diethyl ether followed by centrifugation of the resulting precipitate.

A solution phase synthesis is also within the scope of the present invention. This method provides a method of synthesizing *bis* peptides comprising the steps of:

1) providing a bis-aa or bis-peptide fragment containing a mixture of bis-aa and naturally occurring aa. with an unprotected leading edge amine and a protected trailing edge carboxylic acid;

2) providing a bis-aa or bis-peptide fragment containing a mixture of bis-aa and naturally occurring aa with a protected leading edge amine and an activated ester;

3) coupling the two fragments in solution;

4) isolating the synthesized bis-peptide;

5) removing the leading edge amine protecting group or the trailing end carboxylic acid protecting group; and

6) repeating steps 1,2,3,4 to achieve the desired chain length;

where the *bis* peptide synthesized in the above manner has at least two contiguous *bis* amino acids, and a rigidification step is carried out either after step 3 or after detachment of the *bis* peptide from the solid support. Optionally, the method further comprises the step of modifiying or adding a functional group, after step 3. Macromolecules comprised of 2, 3, 5, 10, 15, 20, 25, 50, 100, 200, 300, or 500 units synthesized by the either of the above methods (solid support or solution phase) are contemplated, with any mixture of monomer units, including mixtures of *bis* amino acids and naturally occurring amino acids.

Isolation of the *bis* peptide would be the same as described above. As used herein, the term "coupling" means to mix an unprotected amine together with an activated ester in solution and waiting for minutes to hours while the amide bond is formed. The amine attacks the activated ester in a bimolecular reaction and forces out the strong leaving group. The result is the formation of a strong, stable amide bond.

The monomer classes of formulas (15) and (16) would be synthesized starting from a suitably protected 4-oxo-pipicolic acid intermediate described in the synthesis of the pip4 monomer class and annulated in the same way as in the pro4a synthesis

followed by conversion of the ketone to an amino ester using a Bucherer-Bergs reaction or a modified Corey-Link reaction.

The monomer classes of formulas (17) and (18) would be synthesized starting from a suitably protected 5-oxo-pipicolic acid intermediate described in the synthesis of the pip5 monomer class and annulated in the same way as in the pro4a synthesis followed by conversion of the ketone to an amino ester using a Bucherer-Bergs reaction or a modified Corey-Link reaction.

The monomer classes of formulas (19) and (20) would be synthesized starting from suitably protected allyl-glycine and ethynylation on the nitrogen. After a Pauson-Khand reaction and reduction of the resulting olefin, the ketone would converted to an amino ester using a Bucherer-Bergs reaction or a modified Corey-Link reaction.

The monomer classes of formulas (21), (22), (23) and (24) would be synthesized using a diastereoselective imino-Diels-Alder reaction with cyclopentadiene. The resulting disubstituted olefin would be hydroborated, and the resulting alcohol would be oxidized to a ketone. The ketone would converted to an amino ester using a Bucherer-Bergs reaction or a modified Corey-Link reaction.

The base-promoted epimerization of diketopiperazines has been studied extensively[43]. It was thought that incubation of scaffolds in base to promote intramolecular aminolysis might cause epimerization and loss of the carefully crafted stereochemical structure. It has now been found that in the reverse phase C_{18} chromatograms of fully rigidified scaffold, traces of compounds with identical molecular weights to the scaffolds appear, but with slightly different retention times. It is thought that these are diastereomeric scaffolds. However they collectively represent less than five percent of the overall material, and could originate from stereochemical impurities in the *bis*-amino acid building blocks. The absence of significant amounts of diastereomeric impurities demonstrates that diketopiperazine epimerization under the 20% piperidine/DMF conditions is much slower than intramolecular aminolysis.

Synthesis of functionalized building blocks

Given the *bis*-amino acids that are disclosed above, it is straightforward to uniquely functionalize the two ends of any poly-*bis*-peptide. To

display more than two functional groups, monomers will be developed that not only contribute to the shape of a scaffold, but also display a selected functional group in the same way that a natural amino acid displays its functional side chain. A general approach to this would be to *a*-alkylate any of the N-protected amino ester ketones just prior to the Bucherer-Bergs reaction. In the prototypical *pro4a* monomer class, the intermediate ketone will be *a*, *a* dialkylated to create a monomer that forms turns in poly-*bis*-peptides and displays an additional functional group (Figure 9).

EXAMPLES

The following examples are intended to illustrate the invention and should not be construed as limiting the invention in any way.

Synthesis of the pro4 monome class

EXAMPLE 1

These monomers were chosen as prototypes because they are capable of forming macromolecules shaped like rods, circles, figure-eights. etc. (Figure 4) and because they can all be made from inexpensive, commercially available trans-4-hydroxy-L-proline 5. The first member of this class **1** was synthesized on a 1.8 gram scale in nine steps with an overall yield of 20% (Scheme 1).

The synthesis began by protecting the amine of 4-hydroxyproline **5** as a benzyl carbamate (*N*-Cbz) followed by oxidation of the secondary hydroxyl to form the ketone **6**. The carboxyl group was then protected as a *tert*-butyl ester to form **7**. A Bucherer-Bergs reaction[31] was carried out to install a quaternary stereocenter and form the diastereomeric hydantoins **8** and **9** with a diastereoselectivity[32] of 5:1. Using a single chromatographic column, 16.5 grams of pure diastereomeric hydantoin **8** was isolated from a 20 gram mixture of **8** and **9**. The hydantoin **8** was then hydrolyzed using a mild, two-step procedure developed by Rebek and co-workers [22] and the amino acid 10 was isolated in excellent yield. The free amine was then protected as the 9-fluorenylmethyl carbamate (Fmoc) and the carboxylate converted to the methyl ester to form the fully protected building block **11**. Finally, the *tert*-butyl ester was deprotected with trifluoroacetic acid and the building block with its free carboxylic acid is used without further purification in solid phase couplings.

The minor hydantoin **9** has been carried to within one step of the building block **2**:*pro4(2S4R)*. The reported yields are unoptimized (Scheme 2).

EXAMPLE 2

Synthetic access to the two additional stereoisomers of the *pro4* monomer class has been established. Using a controlled epimerization procedure[34], 30 grams of *trans*-4-hydroxy-(*L*)-proline **5** were converted to the diastereomer *cis*-4-hydroxy-(*D*)-proline **14** with 57% isolated yield. By carrying this material through the synthesis described in Scheme 1, synthesis of the other two enantiomers of the *pro4* building block class 3:*pro4(2R4R)* and 4:*pro4(2R4S)* can be accomplished. (Scheme 3)

Synthesis of scaffolds

EXAMPLE 3

Pure three-mer scaffolds and five-mer scaffolds with extended rod-like structures in useful amounts have been synthesized. Three units of building block **1**:*pro4(2S4S)* (Sequence 1) were assembled to form the molecular rod **19** using sequential solid phase synthesis on a 46 μmole scale (Scheme 4). The synthesis took place on an AM resin with a Rink Amide linker available from Novabioehem. Each building block was activated as the 1:*pro4(2S4S)*-hydroxy-7-azabenzotriazole (HOAt) ester [23] and quantitative coupling to the previous building block was achieved in less than 10 mm; a surprising result given the apparent hindered nature of the nucleophile. After coupling three monomers, an Fmoc-Tyr(*t*-Bu)-OAt residue was coupled to increase the hydrophobicity of the final scaffold so that it would bind to a C_{18} column. After removal of the tyrosine Fmoc group, the amine terminus rapidly attacked the adjacent methyl ester to form a diketopiperazine **18** (indicated in the sequence as *cyclo*-(Tyr)). The flexible oligomer **17** was cleaved from the resin and its mass was confirmed by reverse phase liquid chromatography with mass spectrometry (RP-LCMS). The carboxybenzyl (Cbz) groups were then removed by hydrogenolysis to obtain **18**. The flexible oligomer **18** was converted into the rigidified scaffold **19** through the parallel formation of two diketopiperazine [24] rings by exposure to 20% piperidine/DMF over 48 hours at 4 °C.[25] The product precipitated from the 20% piperidine solution and filtration provided 5 mg of **19** (~15% yield based on resin loading). The scaffold **19** was soluble in water to more than 5 mg/ml and stable in

neutral and acidic aqueous solution at room temperature for more than three weeks. A previous three-mer synthesis using alanine in the place of tyrosine resulted in a scaffold that was so polar that it refused to bind to a C_{18} column in pure 0.1% TFA/H_2O and eluted in the flow-through. Solubility was measured by dissolving the compound in 0.1% TFA, centrifuging it, injecting the solution into an HPLC and integrating the resulting peak relative to a tyrosine standard. For more accurate solubility measurements sedimentation equilibrium can be used to determine the poly-*bis*-peptide molecular weight in solution [26].

The predicted structure of **19** is consistent with the solution structure. To construct a model of the scaffold **19**, an *in vacuo* conformational search was carried out using the AMBER95 [27] force field within the molecular mechanics package MOE.[28] The conformational search revealed a cluster of five lowest energy conformations all within 0.4 kcal/mol of each other separated from the next highest energy cluster by a gap of 2.2 kcal/mol. Calculating the populations of the 24 lowest energy conformations using the Boltzmann equation suggests that the molecule will spend more than 95% of its time collectively in the five lowest energy minima. A superposition of these predicted lowest energy conformations reveals that rings B, C, D, E, F and the folded tyrosine conformation are identical. The differences between the five conformations involve combinations of rotamers around the C2-C3 bond, rotamers around the tyrosine OH bond and two envelope conformations of ring A. The 2D ROESY spectra display cross-peaks consistent with the predicted rigid B through F ring system. The NMR data are more consistent with ring A existing predominately in the single envelope conformation based upon a strong cross-peak between 4H and 10H and a weak or non-existent cross-peak between 4H and 10H.

EXAMPLE 4

To demonstrate the generality of this synthetic approach, the five-mer scaffold **20** (Figure 5) was synthesized in a similar fashion to scaffold **19**. The resin (49 mg, 31.4 umol loading) was first charged with an Fmoc protected tyrosine residue and then five cycles of coupling with monomer **1**:*pro4 (2S4S)* were performed. Roughly 13 mg of product resin was removed and subjected to the TEA cleavage conditions. The Cbz groups were removed and the scaffold was rigidified by exposure to 20% piperidine/DMF over 24 h. In this case, 3 mg of the scaffold 20 (~33% from

initial resin loading) was isolated by precipitation with ether and centrifugation. After all of these manipulations, this unpurified material was highly homogeneous (Figure 5), and HPLC-MS analysis confirmed that the major peak has the expected mass. This material was soluble in 10% D_2O/H_2O at 5 mg/ml. The *in vacuo* minimum energy structure suggests that the spiro-fused ring structure forms a narrow left handed helical rod with approximately four residues per turn and a pitch of ~20Å.

The sequences of four of these scaffolds are shown (Sequence 2, Figure 6). These particular sequences form interesting shapes that in three cases create close contacts between monomers that are widely separated in sequence. Hundreds of crystal trials using a few milligrams of material[40] can be set up with protein crystallization methods. With 5 mg of a scaffold, a 500 μl solution at 10 mg/ml concentration can be prepared and used to set up 250 2 μl sitting drops for crystallization using the vapor diffusion technique. The solution structures of the compounds **21**, **22** and **24** where predicted close contacts are expected to give rise to specific ROESY cross-peaks can also be determined. Each synthesis will be performed on an Applied Biosystems 433A peptide synthesizer. This instrument is capable of performing syntheses on a 20 μmol scale, requiring only 5 equiv. of building block per coupling (~60 mg of *bis*-amino acid/coupling) providing between 7 mg and 16 mg of the *bis*-peptides **21** and **24** respectively (conservatively assuming 33% recovered yield from resin).

In the event that NMR structure determination is hampered by overlapping signals, ^{15}N and ^{13}C can be site-selectively incorporated to simplify NMR structure determination. Incorporation of ^{15}N and ^{13}C isotopes into proteins is a common technique used to enhance spectral resolution in the determination of protein structures by NMR [29]. These isotopic labels can be used in the Bucherer-Bergs reaction through the use of inexpensive ^{15}N labeled ammonia and ^{13}C labeled potassium cyanide. To further facilitate NMR structure determination, ^{15}N labeled and unlabeled *bis*-amino acids can be mixed at the point where coupled to solid support. This allows rapid assignment of resonances to positions within the sequence by the relative NMR signal intensity within the ^{15}N spectra.

Improvements in solid-phase assembly techniques now permit routine synthesis of long (>40 residues) complex peptides [30]. However, some peptide

syntheses experience difficult couplings when they are elongated through residues 12-20 of their sequences [31]. These difficult couplings are believed to be due to β-sheet formation on solid support. The monomers of the present invention should not be capable of forming β-sheets. Difficult couplings can be addressed by using additional couplings, changing solvents, increasing the temperature and by using more active coupling reagents [31]. Switching to different resins and lower loading resins can also help to avoid coupling problems [31].

Increasing the length of the scaffolds is expected to have a minimal impact on the "rigidification phase" of scaffold synthesis (Figure 3). The kinetics of diketopiperazine closure in scaffolds of arbitrary length are readily modeled. Assuming that every ring closes independently and with the same rate constant, the function that describes the formation of fully closed product is $P_n(t) = e^{-nkt}(e^{kt} - 1)^n$ (Figure 7). In this equation 'n" is one less than the number of building blocks and "k" is the rate constant of diketopiperazine closure. The equation predicts that 532 min is required to achieve 99.9% yield of a fully rigidified 2-mer scaffold-and only 932 mm to achieve the same yield of a fully rigidified 51-mer scaffold. If longer scaffolds do not completely rigidify, as indicated by LCMS analysis of the final product, tandem-mass-spectrometry can be used to fragment the scaffold at any single amide bond. This will allow pinpointing slow closing diketopiperazines and acceleration of their closure by replacing their methyl esters with more active esters such as ß-fluoro-ethyl esters or electron withdrawing group substituted benzyl esters.

Synthesis of the pro3 monomer class

EXAMPLE 5

Trans-3-Hydroxy-(*L*)-proline **39** is commercially available from Acros at $19/gram (2000/2001 catalog) and is the starting point for two diastereomeric members of the *pro3* monomer class. First the carboxylate of **39** will be converted to the methyl ester **40** via a Fischer esterification. Next, the amine will be protected using the 9-phenyl-fluorenyl (PhF) group [32] to obtain **41**. The alcohol will then be oxidized to the ketone **42** using a TPAP oxidation. This sequence is similar to that used by Kamenecka and co-workers in their enantioselective synthesis of 3-substituted prolines [33]. The N-PhF group will protect the a-carbon from epimerization. A Bucherer-Bergs reaction is then carried out to form the two

diastereomeric hydantoins **43** and **44**. In the event that only one diastereomer is formed selectively, the two step Strecker/Bucherer-Bergs sequence suggested by Edward [34] can be used to obtain the second diastereomer. In either case, access to both diastereomers **43** and **44** is obtained. The diastereomeric hydantoins can be separated by chromatography as in the *pro4* monomer class or separated by selective crystallization. The two diastereomeric hydantoins will be carried through independently to form two diastereomeric building blocks. If the hydantoins cannot be separated at this stage, the synthesis can continue with the mixture, with separation of the diastereomers at a later stage.

The purified hydantoin **44** will be hydrolyzed using the method developed by Rebek and co-workers [22] to produce the free amino acid **45**. The amine group is then protected as a 2-nitrobenzenesulfonamide [35] (abbreviated "Ns") and the carboxylate converted to the methyl ester **46**. The 2-nitrobenzenesulfonamide group has been developed as a temporary amine protecting group for peptide synthesis [36]. This is a convenient protecting group that can be cleanly removed in minutes with base and ß-mercaptoethanol. Finally, the PhF group is replaced with a Cbz group to form **47** and selectively hydrolyze the methyl ester to the carboxylate to form the completed building block **35**. Hydrolysis of the secondary methyl ester on C2 (Scheme 7) is expected to be selective over the tertiary methyl ester on C3 [35]. Epimerization of the building block during the final base hydrolysis should not be a problem because of the steric strain that would build up as C2 becomes sp^2 hybridized and interacts with the Cbz group.

By carrying the other hydantoin **44** through the same steps described above (Scheme 7), synthesis of the diastereomeric building block 36:*pro3(2S3S)* is possible. An alternative method of synthesis of the *pro3* class is found in Reaction Scheme 14.

EXAMPLE 6

The starting material for 37:*pro3(2R3S)* and 38:*pro3(2R3R)*, the other two stereoisomers in the *pro3* class can be synthesized using an established chemoenzymatic route. Non-fermenting bakers yeast reduction of the known ketoester **48** [37] has been used to synthesize the protected *cis*-3-Hydroxy-(*D*)-proline 39 with greater than 99% ee (Scheme 8). Gellman and co-workers have used a similar bakers

yeast reduction to synthesize the starting material for the synthesis of their Fmoc-AP(Boc) *beta*-amino acid [38]. The building blocks **37**:*pro3(2R3S)* and **38**:*pro3(2R3R)* can be synthesized using the same approach as was used for **35**:*pro3(2S3R)* and **36**:*pro3 (2S3S)* but starting from *cis*-3-Hydroxy-(*D*)-proline.

Synthesis of the pip4 and pip5 monomer classes

EXAMPLE 7

Syntheses for the *pip4* and *pip5* monomer classes borrows much of the chemistry used in the synthesis of the *pro4* class (Scheme 9). The ring expansion on the intermediate **7** using boron trifluoride etherate and ethyl diazoacetate was carried out to form the two keto esters **51** and **52 [39]**. After decarboethoxylation with sodium chloride in wet dimethyl sulfoxide the two ketones **53** and **54** were separated by chromatography and subjected individually to a Bucherer-Bergs reaction. The Bucherer-Bergs reaction on **53** led to two diastereomeric hydantoins **55** and **56** in a ratio of 3:1 that were separated by chromatography.

The stereochemistry of each diastereomer can be determined using NMR and each can be carried through the same final steps as in the synthesis of 1:*pro4 (2S4S)* to produce two members of the *pip4* monomer class. The Bucherer-Bergs reaction on **54** produced another two digatereomeric hydantoins **57** and **58** with a ratio of 3:2. After separation for these two diastereomers and assignment of their stereochemistry individually carried through to form two members of the *pip5* monomer class. The enantiomers of these two monomers will be accessible through the enantiomeric ketone 15.

Synthesis of the hin monomer classes

EXAMPLE 8

Synthesis for the *hin* monomer class begins with stereochemicaily pure N-Cbz protected *L*-tyrosine **59** and uses the oxidative cyclization chemistry developed by Peter Wipf and co-workers[59] to form the bicyclic N-Cbz protected methyl-ester ketone **60**. A Bucherer-Bergs reaction has been carried out on **60** and obtained two hydantoins **61** and **62** with a diastereomeric ratio of 3:1. These will be separated, their stereochemistry determined, and carried through to form two members of the *hin* monomer class. Several other stereoisomers of this class are accessible starting from

D-tyrosine and through the extremely versatile chemistry developed by the Wipf group to alter the stereochemistry of the hydroindole core[59].

EXAMPLE 9

The synthetic approach (Scheme 11, Scheme 12, Scheme 13) builds on the synthesis of the *pro4* monomer class (Scheme 2). The synthesis starts with the formation of the enamine 69 from the intermediate **7**. The enamine will then be carried through an *a, a'* annulation procedure with allyl *ß, ß'*-dibromo-isobutyrate **70**. An acetic acid workup of the annulation reaction should afford **71**. As precedent, an almost identical annulation reaction has been described involving **69** and 2-chloromethyl-3-chloropropiophenone [40] with greater than 80% yield and complete diastereoselectivity. A similar annulation reaction using ethyl *ß, ß'*-dibromo-isobutyrate and the pyrrolidine enamine of cyclopentanone afforded an 80% yield of *endo*-3-carbethoxybicyclo[3.2.1]octan-8-one [41]. The ketone **71** can be carried forward through a Bucherer-Bergs reaction which is predicted to provide a mixture of diastereomers [34]. If the diastereoselectivity is high, the Strecker/Bucherer-Bergs two step procedure used by Edward [34] can be used to obtain the second diastereomer.

Diastereomeric hydantoins **72** and **73** can be separated using chromatography or crystallization and carried through the final steps to obtain both monomers **88**:*pro4a(2S3R4S)NHR* and **89**:*pro4a(2S3R4R)NHR*. To produce the functionalized monomer 88:*pro4a(2S3R4S)NHR* from the hydantoin **72**, the hydantoin will first be acylatee with Boc-anhydride and DMAP to form **74**. Then the allyl ester **74** will be deprotected with palladium and phenyl-silane to obtain **75** and introduce the desired functionality by activating the resulting carboxylate as an asymmetric anhydride and coupling it with a suitably functionalized primary or secondary amine. Any amine component can be introduced that does not interfere with the subsequent steps of building block and scaffold synthesis.

Many amines can be attached to this building block through the amide linkage to form functionalized versions of **76**. Examples are shown in Figure 10. For reactive functionality protecting groups that have been developed for side chain protection in Boc solid phase peptide synthesis were chosen. These can be removed either by hydrogenolysis or by treatment with a strong acid such as

trifluoromethanesulfonic acid (TFMSA). The functional groups include, but are not limited to, those displayed by natural amino acids such as an amine **77**, a guanidine **78**, a thiol **79**, an alcohol **80**, an imidazole **81** and an indole **83**. Also suitable are non-natural side chains, for example, like the dialkylamino pyridine group **82** that could serve as a nucleophilic catalyst, an anthracene group **84** that could be used to construct fluorescent sensors, and a vicinal diamine **85** that could be used to form a catalytic salen metal complex. As described above, a functional group can be any group which provides additional functionality above that provided by the basic monomer unit.

To complete this functionalized monomer **76** will be carried through the hydrolysis of the *bis*-Boc-hydantoin **76**, N-Fmoc protection and methyl ester formation of the amino acid **86** and finally removal of the *tert*-butyl group of **87** to produce the finished building block **88**:*pro4a(2S3R4S)NHR*.

Through access to **15**, the enantiomer of the ketone 7, the other two enantiomers of the *pro4a*, **91**:*pro4a(2R3S4R)NHR* and **92**:*pro4a(2R3S4S)NHR* can be synthesized. Many of the other monomer classes, including the *pip4* class, the *pip5* and the *hin* class (Figure 1) have as intermediates *N*-Cbz *tert*-butyl ester ketones that will be subject to the annulation reaction shown in Scheme 11. These will lead to many other functionalized *bis*-amino acids.

Whereas particular embodiments of this invention have been described above for purposes of illustration, it will be evident to those skilled in the art that numerous variations of the details of the present invention may be made without departing from the invention as defined in the appending claims.

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